Reference: *Biol. Bull.*, **155**: 542–562. (December, 1978)

FEEDING AND DIGESTION IN THREE ENTOSYMBIOTIC GRAFFILLID RHABDOCOELS FROM BIVALVE AND GASTROPOD MOLLUSCS

J. B. JENNINGS AND JAMES I. PHILLIPS

Department of Pure and Applied Zoology, University of Leeds, Leeds LS2 9JT, England, U.K.

The entosymbiotic rhabdocoels show considerable variation in the extent of their adaptations to life within other organisms and this is particularly evident in their nutritional physiology (Jennings, 1971; 1974a). In some species, exemplified in the family Umagillidae by *Syndesmis antillarum* and *S. franciscana* from the gut and perivisceral spaces of echinoids, the diet, gut and digestive physiology are vitually identical with those of free-living flatworms (Jennings and Mettrick, 1968; Mettrick and Jennings, 1969). The symbiotes feed on co-symbiotic protozoa which abound in their habitat; they do not abstract the host's food nor feed on its tissues to any appreciable degree and their presence has no recognizable adverse effects.

In other, more extreme, instances, exemplified in the Fecampiidae by *Kronborgia amphipodicola* from the haemocoel of amphipods, the entire gut is lost and the rhabdocoels apparently feed cestode-fashion by absorption through the body surface (Christensen and Kanneworff, 1964; 1965). The epidermal microvilli are longer and more numerous than in free-living flatworms (Bresciani and Køie, 1970), presumably as an adaptation to this mode of nutrition, and the rhabdocoels cause atrophy of the host's gonads and, eventually, its death.

Most other studies on entosymbiotic rhabdocoels have been morphological or taxonomic; these indicate, however, that while there are probably many intermediate conditions between the umagillid and fecampiid modes of nutrition, the bias lies toward the former because most known species have seemingly functional alimentary systems and a normal epidermis. The resemblance to the umagillid pattern, though, may well be only superficial. A decisive factor in umagillid nutrition is the occurrence of large numbers of co-symbiotic prey organisms; this is a characteristic feature of echinoderms (Hyman, 1955) but is not found to the same extent in molluscs, which are the next most favored hosts of entosymbiotic rhabdocoels. Mollusc-rhabdocoel symbioses are of particular interest, since the monogenetic and digenetic trematodes are believed to have evolved from rhabdocoel-like ancestors (Hyman, 1951; Llewellyn, 1965; Baer, 1971) and the Digenea retain initimate associations with molluscs during their larval stages. Further, both Monogenea and Digenea possess functional alimentary systems as adults; they utilize their hosts' ingesta or tissues as food and show concomitant modifications of digestive physiology (Jennings, 1968). Nothing is known, though, of the nutrition of those rhabdocoels entosymbiotic with molluscs, or of how it compares with that of other rhabdocoels and of larval and adult trematodes. To remedy this, three closely related species from the family Graffillidae, two ento-

symbiotic in bivalves and one in gastropods, have been examined with particular reference to their diet, feeding behavior, gut structure and digestive processes. These features were found to be considerably influenced by the same aspects of the hosts' nutritional physiology and consequently these, too, have been studied and are reported on where relevant.

Materials and Methods

The graffillid species studied were *Paravortex scrobicularíae* (Graff), *P. cardii* Hallez and *Graffilla buccinicola* Jameson. *P. scrobicularíae* was obtained from the intestine and digestive gland of the bivalve mollusc *Scrobicularia plana* (da Costa), collected bimonthly during 1976 and 1977 from mud banks near high water mark in the estuary of the River Esk, Whitby, Yorkshire; *P. cardii* was taken from the digestive gland of the bivalve *Cerastoderma edule* (L.), collected in October and November 1975 from around low water mark on sandy shores at Spurn Head, Yorkshire and at irregular intervals during 1975–77 from similar habitats at Plymouth, Devon; and *G. buccinicola* was obtained from the digestive glands of the gastropods *Buccinum undatum* L. and *Neptunea decemcostata* (Say). *B. undatum* was collected during December 1977 and January 1978 from 20 to 30 m of water off the Isle of Cumbrae, Scotland and *N. decemcostata* in July 1977 from 20 m in Cobscook Bay, Eastport, Maine.

Specimens of *S. plana* and *C. edule* were fixed in marine Bouin's fluid or 10% neutral formalin as they were collected during the intertidal period, for subsequent histological examination of the rhabdocoels *in situ* within their hosts. Section of the adductor muscles facilitated penetration of the fixatives. Others were dissected in sea water and the rhabdocoels fixed separately in marine Bouin's, 10% neutral formalin, Susa, 90% ethanol or Flemming's fixative. Living bivalves were maintained in circulating sea water in Leeds for periods of up to four months and used in feeding experiments in which suspensions of rice starch grains, yeast cells stained with Congo red, or bacteria isolated from sea water and cultured on seawater-nutrient agar were introduced into their inhalent currents. Such fed bivalves were then either fixed in marine Bouin's or 10% neutral formalin, or dissected and the rhabdocoels fixed, at progressive intervals up to 24 hr after feeding. Parallel observations were made on living rhabdocoels from fed hosts, examined by bright field, dark ground and polarized light illumination systems or after intravital staining with 0.05% seawater solutions of Neutral red or Janus green.

The gastropod *B. undatum* was maintained in circulating sea water until required; individuals were then dissected and either the entire digestive gland or rhabdocoels removed from this fixed, as above. Only preserved material from *N. decemcostata* was available; this was an intact digestive gland fixed in marine Bouin's six hr after collection of the gastropod, and was kindly supplied by Mr. Carter Newell of the Cobscook Bay Marine Laboratory.

Histological and histochemical observations on the gut contents, gut structure, food reserves, sequence of digestion of the natural and experimental foods, and the identity, origin and sites of action of the digestive enzymes were made on 4 or 8 μm serial sections of appropriately fixed materials and on whole mounts of indi-

vidual flatworms. Stains and techniques used included: Ehrlich's haematoxylin and eosin; Heidenhain's haematoxylin and metanil yellow; Mallory's triple stain; Nuclear Fast red; Feulgen's reaction for DNA; Steedman's Alcian blue method for acid mucopolysaccharides; the periodic acid-Schiff (PAS) reaction for carbohydrates and acid mucopolysaccharides; Best's stain for glycogen; the Sudan IV and Oil red O methods for lipids; Twort's modified Gram stain; the Cyanol blue method and a modified diaminobenzidine technique (Phillips, 1978) for haemoglobin; Perl's method for inorganic iron and the alizarin-ammonium hydroxide method for inorganic iron and calcium.

For histochemical studies of digestive enzymes, fixation was at 1° C in 10% formalin buffered with phosphate to pH 7.0. Whole mounts, serial sections prepared after dehydration in graded acetones and impregnation in paraffin wax (melting point, 39° or 45° C) and frozen sections were treated by the indoxyl acetate method for non-specific esterases (Holt, 1958), the L-leucyl β-naphthylamide hydrochloride method for arylamidases (Burstone and Folk, 1965), the naphthyl AS-BI phosphate methods for acid and alkaline phosphatases (Burstone, 1958), the post-coupling 6 bromo-2-naphthyl-β-D-glucopyruronoside (glucuronide) method for β-glucuronidase (Pearse, 1972), the 6 bromo-2-naphthyl-β-D-galactopyranoside method for β-galactosidase (Pearse, 1972) and the naphthol AS-nonanoate method for lipase (Abe, Kramer and Seligman, 1964). Esterases demonstrated by Holt's method were characterized further, using specific inhibitors and activators and following procedures and interpretations given by Pearse (1972) and Hassall and Jennings (1975).

Positive controls for the enzyme studies consisted of simultaneous processing of appropriate mammalian and molluscan tissues; negative controls consisted of heat inactivated sections (held at 90° C for 2 min before processing) and the omission of specific substrates from incubation media.

Observations and Results

Paravortex scrobicolariae

70.4% of the *Scrobicularia plana* examined contained *Paravortex scrobicolariae;* the overall mean infection rate was 6.1 rhabdocoels per host with a range of 0 to 77 and a variance of 0.027. No seasonal fluctuations in the rhabdocoel population could be discerned and adults with embryos in all stages of development were found throughout the year. The species is hermaphrodite and viviparous; thin-walled capsules usually containing two developing embryos and a mass of yolk cells are given off from the female atrium into the mesenchyme. Mature individuals contain up to 40 capsules with embryos in different stages of development (Fig. 1). Fully developed embryos, resembling tiny immature adults, were seen to leave their capsules and move freely about the parental mesenchyme before passing through the gut wall into the intestine (Fig. 3). Birth is presumably via the mouth, in the primitive turbellarian fashion, but this was not observed.

Structure of the gut. The gut in *P. scrobicolariae* has the morphology typical of dalyellioid rhabdocoels (Fig. 2). The anterior subterminal mouth opens ventrally through a small ciliated buccal cavity into a doliiform muscular pharynx

which lacks both intrinsic and extrinsic gland cells. A short (50–60 μm) ciliated oesophagus, composed of a single layer of cuboidal cells, links the pharynx with the simple saccate intestine. This occupies about two-thirds of the body length and is median in immature individuals, but in older ones is compressed and displaced laterally or dorsally by the vitellaria and developing embryos. The intestinal wall, or gastrodermis, (Figs. 2, 3, 7) is a single layer of monotypic broadly columnar cells, 75 to 100 μm tall and 30 to 50 μm wide, with basal nuclei and cytoplasm usually containing up to four large vacuoles. Gland cells of the type common in other Turbellaria are absent.

The gastrodermal vacuoles contain a clear proteinaceous fluid in which there is usually suspended a variety of spherical or granular inclusions. Most of these resemble in appearance and histological and histochemical reactions inclusions in the digestive cells of the bivalve host. In particular, many show the intense positive reactions for inorganic iron and calcium which are a characteristic feature of host digestive cell inclusions at certain stages of the host digestive cycle (Figs. 4, 5). Other, smaller inclusions very near the limit of resolution of the optical microscope show birefringence when viewed by polarized light.

The vacuoles may so distend the gastrodermis that opposing cells come together and occlude the lumen at irregular intervals, resulting in an intestinal structure dominated by large intra- and intercellular spaces. The latter, and the entire lumen when present as an uninterrupted entity, often contain the same materials as the intracellular vacuoles. This is particularly common in rhabdocoels from hosts fixed at low water.

The early embryonic gut consists of a pharynx and an undifferentiated mass of endoderm and yolk. By the time embryos are ready to leave their capsules and invade the parental mesenchyme (indicated by their continual rotation within the capsule by the beating of the epidermal cilia) the gut is organized into the adult form. The intestinal cells are vacuolated; the vacuoles have inclusions, but these are restricted usually to minute birefringent granules of the type seen in adult gut cells.

The food and feeding behavior. The diet, feeding behavior and digestive physiology of *P. scrobicularíae* are all greatly influenced by the feeding and digestive cycles of the host bivalve, especially the regular cycle of growth, food uptake, intracellular digestion, and disintegration undergone by the cells lining the distal tubules of its digestive gland. These cycles, which in turn are related to tidal rhythms and the duration of submergence at high water, must therefore be described. Only such detail as is necessary for understanding of the nutritional physiology of *P. scrobicularíae* will be given, however, as the cycles conform to the general pattern characteristic of intertidal bivalves as interpreted by Owen (1966; 1972; 1974) and reviewed by Purchon (1977). In particular, the digestive cycle closely resembles that described in *Lasaea rubra* by McQuiston (1969), who extended and reinterpreted the original observations on this species made by Morton (1956) and Morton, Boney, and Corner (1957).

Feeding and digestion in the host Scrobicularia plana. S. plana lives 15 to 20 cm deep in mud near high water mark and is submerged for 2 to 3 hr in each 12 hr tidal cycle. Specimens were occasionally found near mid-tide level or lower; these, obviously, were submerged for longer periods per cycle and did

not show as clearly the cyclical changes in the digestive gland observed in those from the upper shore.

The bivalve is primarily a deposit feeder (Hughes, 1969) but is also capable of sustained suspension feeding. In the laboratory this facilitated the introduction of test foods into the inhalent current. The organic deposits forming the natural food of the population sampled were rich in bacteria, diatoms, protozoa, unicellular algae, and organic detritus; virtually all of these items contained much loosely bound iron and calcium which were easily demonstrated by the Perl's and alizarin techniques and formed, therefore, convenient labels of ingested natural foods throughout the digestive cycle.

The bivalve feeds while submerged at high water. Specimens collected as the tide receded, or from appropriate laboratory simulations using test foods, had well developed crystalline styles and full stomachs whose organic contents showed extensive extracellular digestion. The laboratory-fed specimens showed that this is effected by non-specific esterases, A- and C-esterases, β-glucuronidase, and arylamidases. Silt particles, and yeast cells or starch grains taken in excess by laboratory-fed specimens, were being passed into the intestine, which was filled with such rejected materials being transported by ciliary action to the anus. Semi-digested soluble and finely particulate organic materials were passing from the stomach through the main and secondary ducts of the digestive gland into the blind-ended distal tubules; the amount passing increased until around the time of low water, when the stomach was usually empty. The style in such specimens varied considerably, being absent from some and moderately to well-developed in others.

Shore-fixed specimens showed that there is some absorption by the unciliated epithelium of the secondary ducts during the passage of semidigested materials into the tubules, the epithelial cells developing transient reactions for iron and calcium.

The cycle of growth, intracellular digestion and disintegration undergone by the digestive cells of the tubules was found to be diphasic; all cells within a given tubule are in synchrony, but at any one time approximately half the tubules are in one stage of the cycle, while the other half are in the opposing stage. A complete cycle from juvenile stage to total disintegration occupies 24 hr, the stages are correlated with tidal (and hence feeding) cues and the disintegration phase coincides, roughly, with low water.

The mature digestive cells lining the fully developed tubules at high water contain many spherical vesicles, 2 to 10 μm in diameter, which show strong positive reactions for acid phosphatase, β-glucuronidase, β-galactosidase, non-specific esterases, A- and C-esterases, lipase and arylamidases. These are presumably primary lysosomes. Materials entering the digestive tubules from the stomach are rapidly taken up by these cells; occasionally fragments of algal cells, starch grains or intact bacteria could be recognized in the cells, but usually the engulfed materials were homogeneous, indicating extensive extracellular digestion in the stomach and tubule lumen. Vesicles 0.2 to 10 μm in diameter and showing in naturally-fed specimens strong reactions for iron and calcium appear in the digestive cells in increasing numbers. These are obviously newly formed food vacuoles or phagosomes; they show at first only moderate enzymic activity equivalent to that shown by the food as it enters the digestive gland from the

stomach but quickly develop the intense reactions seen in the primary lysosomes. These include reactions for lipase and β-galactosidase, enzymes not demonstrated in the stomach. Eventually all vesicles in mature digestive cells show strong enzymic reactions, and the majority also show reactions for iron and calcium, indicating fusion of the primary lysosomes and phagosomes to form heterolysosomes within which intracellular digestion proceeds. Immature cells in other tubules show all types of vesicles, usually in smaller quantities and with less intense reactions for iron and calcium.

Intracellular digestion in the mature cells is completed around the time of low water; the heterolysosomes have shrunk as digested products are absorbed from them, but they contain one or more residual bodies staining intensely for iron and calcium and still showing much enzymic activity. Such cells disintegrate and the recognizable fragments, dominated by the residual bodies, pass down the tubules, through the secondary and main ducts (Fig. 5) and enter the stomach. A minority may pass directly into the intestine. Developing cells lining tubules in the opposing phase often still show intracellular digestive activity; these cells, of course, rapidly become the mature cells which will undertake the bulk of the intracellular digestion of the food to be taken during the next highwater feeding stage.

Tubules which have lost their lining of digestive cells are at this stage balloon-like structures with membranous walls. The source of the replacement epithelium was not seen; the junctions of the tubules and secondary ducts are lined by small cuboidal basophilic cells and these might give rise to the next generation of digestive cells.

Diet and feeding behavior in P. scrobiculariae. *P. scrobiculariae* feeds partly on semi-digested components of its host's food, which it abstracts while this is passing from the stomach into the digestive gland, and partly on the residual bodies and cellular debris released by the disintegration of mature host digestive cells at the end of their digestive cycle.

At high water the rhabdocoels are to be found almost exclusively in the anterior portion of the host's intestine, where they occur either singly or in groups of up to 10 or more (Fig. 6). The intestine is usually filled with silt particles, sand grains and other particulate materials, bound into a column with mucus, which have been rejected by the stomach's sorting mechanism and are being moved by the cilia of the intestinal epithelium towards the anus for defaecation. The rhabdocoels lie between the column and the intestinal epithelium and are always orientated with their ventral surfaces applied to the latter.

Previous accounts have described *P. scrobiculariae* only from the intestine (Villot, 1879; Wahl, 1906). Examination of hosts collected on the ebb tide, however, revealed that many of the rhabdocoels migrate anteriorly at this time from the intestine and pass through the main ducts of the digestive gland into the secondary ducts (Figs. 4, 5). A few penetrate almost into the digestive tubules proper but none were ever seen actually within the expanded distal portion of the tubules. Similarly, they were only rarely seen in the stomach, except around the intestinal groove and the bases of the main ducts of the digestive gland; such individuals were presumably in transit to or from the gland.

The rhabdocoels remain in the secondary ducts over low water but migrate

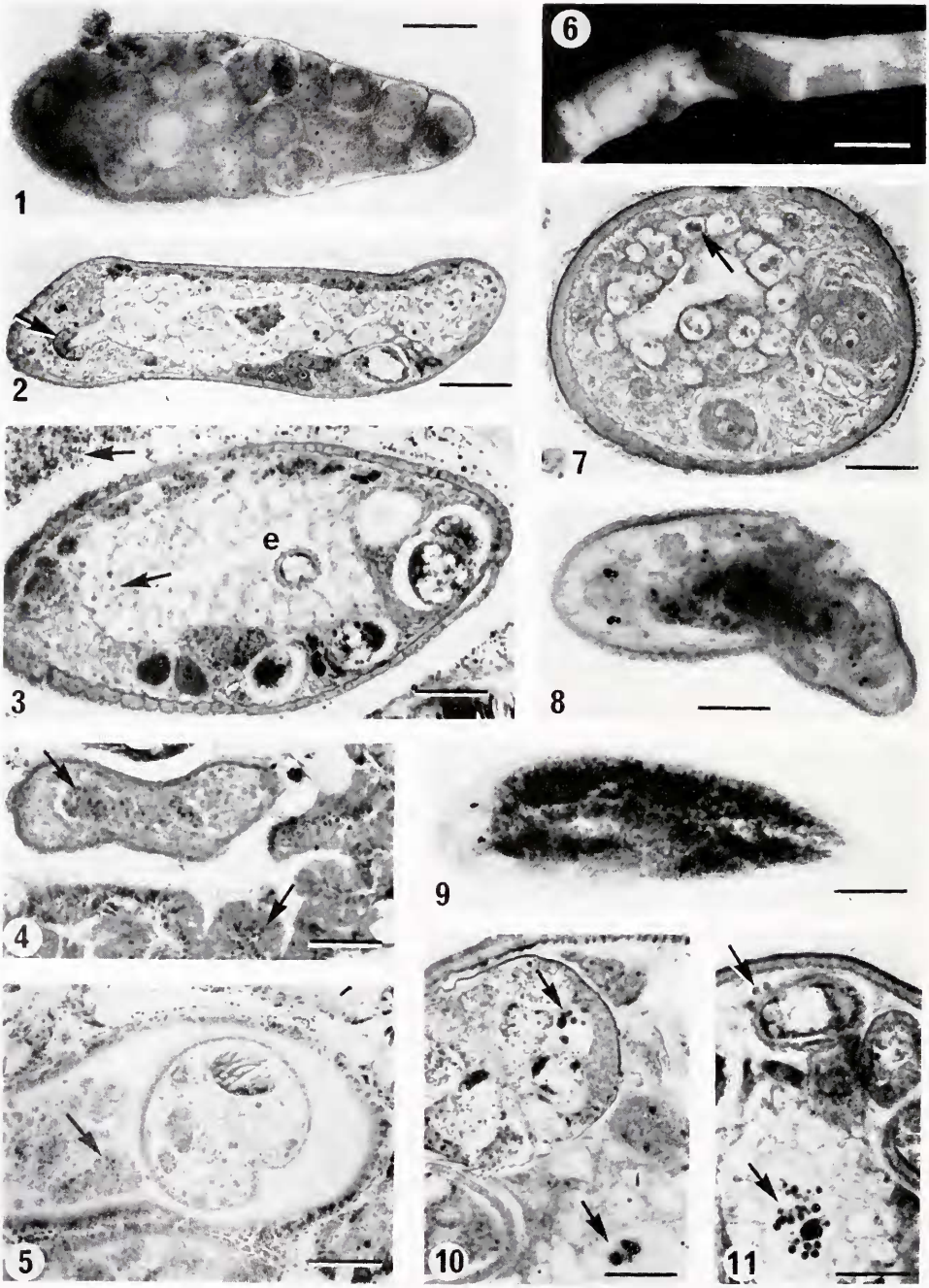

FIGURE 1. *Paravortex scrobiculariae*. A whole mount preparation treated by the cyanol blue method and showing a strong reaction (black) for haemoglobin around the brain and pharynx at the broad anterior end of the animal. The specimen contains many pairs of

back to the anterior intestine during the flood tide. While in the digestive gland they ingest materials present in the ducts; in the earlier part of their stay this consists mainly of the host's own semi-digested food passing up the ducts to the digestive tubules. This was proved by the demonstration of intact and partially digested yeast cells, starch grains and bacteria in the gut cells of *P. scrobiculariae* from the ducts of *S. plana* fed on these materials during a simulated highwater submergence (Fig. 7). In the later part of their stay, however, the ducts contain

---

embryos within capsules in the mesenchyme; some, with prominent eyes, are at an advanced stage of development. Rupture of the specimen during preparation allowed partial extrusion of an early embryo (top left) through the body wall. The scale bar represents 0.25 mm.

FIGURE 2. *P. scrobiculariae*. A near sagittal section through a mature individual showing the pharynx (arrow), oesophagus, intestine and the intestinal wall or gastrodermis. The section is stained with Heidenhain's haematoxylin and metanil yellow; the scale is 0.2 mm.

FIGURE 3. *P. scrobiculariae*. A transverse section through the middle of the body showing an embryo (e) lying within the parent's intestine and others still in their capsules in the mesenchyme. The specimen was fixed *in situ* in the intestine of a *Scrobicularia plana* fed in the laboratory on a dense suspension of yeast cells; these (arrows) can be seen in the lumen of the host's intestine and within the intracellular vacuoles of the rhabdocoel's gastrodermis. The section is stained with Heidenhain's haematoxylin and metanil yellow; the scale is 0.1 mm.

FIGURE 4. *P. scrobiculariae*. An oblique longitudinal section through an immature specimen lying at the junction of a secondary duct and a digestive tubule, within the digestive gland of a *Scrobicularia plana* fixed at low water. The digestive epithelium of the tubule consists of mature cells (lower right) which contain iron-rich vesicles (arrow); similiar vesicles can be seen in the rhabdocoel's intestine (arrow). The section was treated by Perl's method for inorganic iron and is counterstained with Nuclear Fast red. The scale is 0.5 mm.

FIGURE 5. *P. scrobiculariae*. A transverse section through an individual lying in the lower part of a secondary duct of the digestive gland of a *Scrobicularia plana* fixed at low water. The duct contains, in addition to the rhabdocoel, cellular debris and iron-rich vesicles (arrow) from disintegrating digestive cells. Preparation and staining of the section were the same as for Figure 4; the scale is 0.5 mm.

FIGURE 6. *Scrobicularia plana*. The anterior portion of the intestine of an individual fixed at simulated high water and containing many *P. scrobiculariae* which are lying between the central core of silt particles and the intestinal wall. The rhabdocoels are all oriented with their ventral surfaces to the wall and hence their eyes, which lie dorsally just below the body wall, are not visible. The scale is 2.0 mm.

FIGURE 7. *P. scrobiculariae*. A transverse section through a mature individual, with well developed ovaries, fixed *in situ* within a secondary duct of a *Scrobicularia plana* fed on yeast cells during a simulated high water period. The duct (not shown) contained many intact and partially digested yeast cells; some of these had been ingested by the rhabdocoel and can be seen in intracellular vacuoles (arrow) within the gastrodermis. The section is stained by Twort's modified Gram stain; the scale is 0.1 mm.

FIGURE 8. *P. scrobiculariae*. A whole mount preparation of an individual dissected from its host and fixed after being kept for four days without food in filtered seawater. The intestine shows relatively slight reactions for A- and C-esterases, as compared with the specimen shown in Figure 9, and no reactions are visible in the intestines of the embryos. The preparation was treated by Holt's (1958) method for non-specific esterases, modified for the demonstration of A- and C-esterases as described by Pearse (1972) and Hassall and Jennings (1975). The scale is 0.25 mm.

FIGURE 9. *P. scrobiculariae*. A whole mount preparation of an individual fixed immediately after removal from a secondary duct of the digestive gland of a naturally-fed *Scrobicularia plana*. The intestine shows a high degree of reactivity to the technique for A- and C-esterases. Treatment of the preparation was the same as for that shown in Figure 8; the scale is 0.25 mm.

only the residual bodies and cellular debris from the disintegrating host digestive cells; these are passing down the ducts to the stomach and the rhabdocoels ingest quantities of these materials before returning to the intestine. This was apparent from the appearance in the rhabdocoels' intestine and gastrodermal cells of residual bodies staining heavily for iron and calcium (Fig. 4).

Ingestion is effected by a combination of ciliary and muscular activity. Cilia fringing the mouth and lining the buccal cavity sweep small particles and fluid into the mouth; contractions and expansions of the doliiform pharynx supplement the ciliary action and also draw in larger particles such as digestive cell nuclei and residual bodies. Rhabdocoels, isolated from the host and starved for up to 4 days in filtered sea water, fed in this way on yeast and bacterial suspensions but only small amounts were ingested and it was impossible to maintain the animals *in vitro* for any longer period.

The rhabdocoels are very rarely found in any part of the digestive gland during the period of high water and active feeding by the host. Conversely, though, some can always be found in the intestine of an infected host over the mid-ebb to mid-flow tidal period when others are actively feeding in the digestive gland. Such individuals contain little that is recognizable in their intestines apart from a few iron- and calcium-rich residual bodies. It is concluded, therefore, that *P. scrobiculariae* does not necessarily migrate to the digestive gland, and feed, during each tidal cycle but may remain in the intestine over one or more cycles to digest a meal taken earlier.

The host's intestinal contents occasionally include residual bodies from the digestive gland and it is conceivable that *P. scrobiculariae* may ingest these during its stay in the intestine. It is certain that feeding can occur in the intestine under laboratory conditions; when yeast cells, bacteria and starch grains were fed in excess to the host, many passed directly to the intestine and within a few minutes some had been taken up by rhabdocoels lying in the intestinal lumen (Fig. 3). Under natural conditions, though, the host's intestine usually contains very little potential food and it seems likely that feeding normally occurs predominantly, if not entirely, in the ducts of the digestive gland, following migration from the intestine during the period between successive high tides.

Digestion and food reserves. Serial sections and whole mounts of *P. scrobiculariae* fixed after being kept in filtered sea water for 4 days subsequent to their removal from the host showed that the gastrodermal cells contain only small amounts of endogenous enzymes. These are non-specific esterases, A- and C-esterases, acid and alkaline phosphatases, lipase and traces of β-glucuronidase

FIGURE 10. *Paravortex cardii.* Part of a transverse section of an individual removed from a main duct of the digestive gland of a *Cerastoderma edule* fed intermittently on yeast cells over a period of three hours' total submergence. Yeast cells (arrows) can be seen in the intestinal lumen of the adult rhabdocoel and also in the intestine of an encapsulated advanced embryo. The section is stained with Heidenhain's haematoxylin and metanil yellow; the scale is 0.05 mm.

FIGURE 11. *P. cardii.* Part of another transverse section of the same individual used for the preparation seen in Figure 10. Yeast cells (arrows) can be seen in the intestinal lumen of the adult, as before, and also within an embryo's capsule, lying in the space between the capsule's wall and the embryo. The section is stained as for Figure 10; the scale is 0.1 mm.

(Fig. 8). No reactions could be obtained for β-galactosidase or arylamidases. The reactions for the other enzymes named were diffused throughout the cytoplasm and, so far as could be discerned with the light microscope, were not localized within lysosomes of the size and type seen in *Scrobicularia*. The pharynx and oesophagus are devoid of demonstrable enzymic activity, other than acid phosphatase in the intrinsic and extrinsic muscles of the pharynx.

No significant quantitative changes in enzymic activity could be demonstrated in *P. scrobiculariae* fed *in vitro* on starch grains or boiled yeast and bacterial suspensions, irrespective of the time elapsing between feeding and fixation. Increased non-specific esterase activity was found after feeding living bacteria but such activity clearly resided in the ingested food.

In marked contrast, rhabdocoels fixed during their stay in the ducts of the host digestive gland showed greatly enhanced enzymic activities (Fig. 9). These were localized in the inter- and intracellular vacuoles of the gastrodermis and their development coincided with the appearance at the same sites of vesicles and particles recognizable histologically and histochemically as originating either in the host's food or its digestive cells. This indication that the bulk of the enzymes effecting digestion of the food in *P. scrobiculariae* are, like the food itself, derived from the host, was confirmed by the occasional detection of β-galactosidase in the vacuoles. This enzyme was never detected in unfed rhabdocoels.

Enzymic activity continues at this elevated level for as long as visibly recognizable food remains in the gut vacuoles. It then fades to the level seen in unfed specimens and this diminution of activity coincides with the disappearance of reactions for iron and calcium. This latter disappearance implies that the rhabdocoel is capable of breaking down the residual bodies of the spent heterolysosomes of the host; those entering the host's stomach, in contrast, appeared to resist breakdown, but this point could not be confirmed.

Small quantities of lipid occur in the gastrodermis as droplets 0.3 to 1.0 μm in diameter. Larger amounts occur in the vitellaria, but these are obviously destined for inclusion with the fertilized eggs as energy stores for the developing embryos and do not constitute a normal long-term reserve for the adult.

The gastrodermal cells mesenchyme and vitellaria contain significant amounts of glycogen; no quantitative data were obtained but the strong reactions at these sites with the Best's carmine and PAS techniques indicated formation and storage of relatively large amounts.

Nutrition of the embryo. The developing embryo utilizes the lipid reserves of the yolk globules and these are exhausted by the time that the pair of embryos within each capsule are fully developed with eyes, nervous system and an organized gut. As the lipid reserves disappear, tiny birefringent granules accumulate in the vacuoles of the gut cells; these appear to be endproducts of lipid metabolism and are seen in adult gut cells also, but could not be identified. There was some evidence that once the yolk reserves are exhausted, and before the embryos leave their capsule, nutrients are passed from the parental gut through the capsule to the embryos. In a number of instances, inorganic iron was demonstrated in the gut cells of well developed embryos within adults from *Scrobicularia* fixed on the shore. The reaction was diffuse, resembling that seen in the epithelium of the secondary ducts of the host's digestive gland, and could have resulted from materials ingested

by the rhabdocoel being passed directly to the embryos. Similar diffuse reactions for iron were occasionally seen in the gut cells of adults containing such embryos.

Support for this idea of supplementary nourishment of the embryos prior to their leaving the capsule came from the observation of yeast cells and fragments of starch grains, identified by their staining reactions with Twort's modified Gram stain, PAS and iodine, within the capsules and on occasion within the embryonic gut. An identical situation was observed in *Paravortex cardii* (Figs. 10, 11). The capsule wall appears to remain intact in such instances and the mechanism of transfer of soluble and particulate nutriments from the parental gut remains unknown.

Embryos which had reached the parental gut were sometimes seen to have recognizable materials such as residual bodies of host origin and yeast cells in their gut vacuoles, but these could well have been ingested after the embryo had invaded the adult gut. Late embryos released from their capsules in squash preparations swam freely and showed the pharyngeal contractions seen in feeding adults.

Haemoglobin. P. scrobiculariae possesses a red pigment which is concentrated anteriorly around the pharynx and brain and has a diffuse distribution throughout the rest of the body, apart from a slight concentration at the extreme posterior. The pigment was identified as a haemoglobin by its positive reaction to the cyanol blue and diaminobenzidine techniques (Fig. 1). These reactions were obtained in living specimens and others fixed in 10% neutral formalin; pre-heating specimens to 90° C for up to 1 hr did not abolish the reaction, demonstrating that it was not caused by peroxidases or catalases. Exposure of living specimens to carbon monoxide by gently bubbling the gas through the sea water changed the normal orange to red coloration to a permanent, bright, cherry-red but this did not affect the *in vitro* survival rate, treated specimens surviving as long as untreated ones away from the host.

The pigment is absent from the embryos but appears to develop soon after birth, judging from its presence in small immature adults which lack, as yet, any trace of the reproductive system.

In contrast, no reactions for haemoglobin could be obtained in either of the other two species studied.

Paravortex cardii

88.2% of the *Cerastoderma edule* from Plymouth contained *Paravortex cardii;* the overall mean infection rate was 5.7 rhabdocoels per host with a range of 0 to 38 and a variance of 0.03. It was found that 62.5% of those from Spurn Head were infected, with an overall mean infection rate of 1.9 per host, a range of 0 to 26 and a variance of 0.27. Both populations of rhabdocoels resembled that of *P. scrobiculariae* in showing no seasonal fluctuations; *P. cardii,* like *P. scrobiculariae,* is viviparous, and adults containing embryos were found throughout the year.

Diet, feeding, gut structure, digestion and food reserves. The general pattern of nutrition in *P. cardii,* both intrinsically and in relation to its bivalve host, is virtually identical with that described for *P. scrobiculariae* and will not, there-

fore, be considered under separate headings. The only major difference is that *P. cardii* does not show any migration within the gut of its host for feeding purposes but lives permanently in the main ducts of the digestive gland. Previous accounts (*e.g.,* Hallez, 1909; Atkins, 1934) state that *P. cardii* occurs in the stomach of *Cerastoderma edule,* but careful dissection of bivalves killed instantaneously by plunging them into *iso*-pentane cooled in liquid nitrogen shows that the rhabdocoels are, in fact, always located within the main ducts of the digestive gland irrespective of tidal or other factors. Dissection or fixation of living bivalves causes contractions throughout the body which expel many of the rhabdocoels into the stomach and this is presumably the reason for the earlier erroneous reports. Rhabdocoels were only rarely found in the secondary ducts of the digestive gland, however, and this is believed to be due to the small size of the ducts relative to those of *Scrobicularia plana,* which can be up to two or three times wider.

Our observations on feeding and digestion in *C. edule* agree broadly with those by Owen (1972) on this bivalve and hence will be reported only briefly. The specimens studied came from near low water mark and were usually submerged, therefore, for a much larger proportion of each tidal cycle than *S. plana.* Consequently, feeding and digestion were found to be virtually continuous and they did not show the cyclical features seen in the latter species.

Specimens fixed on the shore usually had full stomachs in which diatoms, other unicellular algae and protozoa predominated. Organic detritus, so abundant in the stomach of *S. plana,* was present in much smaller amounts, but both this and the food organisms contained small but demonstrable quantities of iron, which again formed a convenient marker of subsequent digestive processes. It was apparent from these specimens, and others in laboratory simulations, that the more or less continuous feeding is accompanied by extracellular digestion in the stomach and movement of both intact and semi-digested food into the digestive gland. Here uptake of food by the digestive cells and the completion of digestion intracellularly occur by processes very similar to those seen in *S. plana.* The range of enzymes demonstrated in the stomach contents and primary lysosomes of the digestive cells was the same as in that species and a few iron-rich residual bodies were again visible endproducts. The digestive cells, however, take up materials in different amounts and at different rates; synchronous stages within tubules or sets of tubules were never seen and the residual bodies are usually eliminated by abstriction. Cells which have voided their residual bodies appear to regenerate; only rarely was complete degeneration and shedding of digestive cells seen.

P. cardii ingests the intact and partly digested components of the host's food as they pass through the main ducts into the digestive gland. Unaltered mucus, from either the original food-string of the host or the stomach, was often present in the ducts and this, too, was ingested by the rhabdocoel. Residual bodies, some clearly "labeled" with inorganic iron, from the digestive gland are also ingested as they pass down the ducts to the stomach.

The gut structure is very similar to that of *P. scrobicularlae,* except that the gastrodermal cells are somewhat smaller, measuring 50 to 60 μm by 15 to 20 μm, and do not tend to occlude the intestinal lumen. Starved specimens showed slight to moderate amounts of non-specific esterases, A- and C-esterases, acid and alkaline phosphatases, lipase and β-glucuronidase in their gastrodermal cells. As in the case

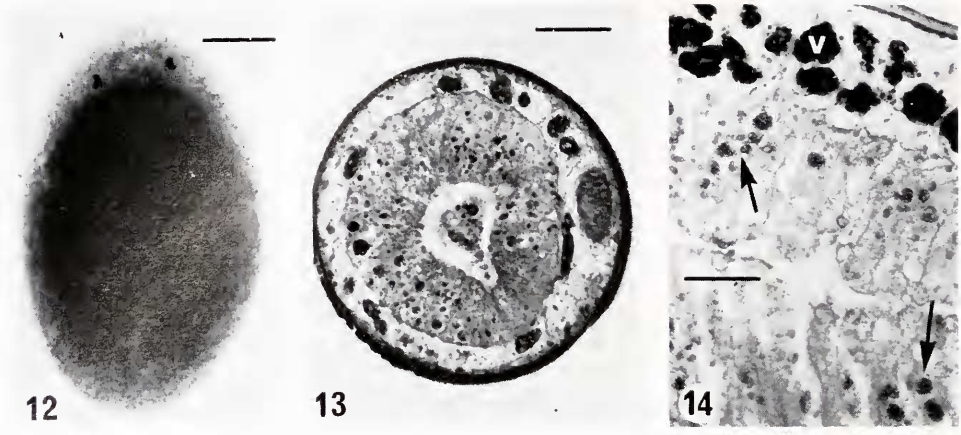

FIGURE 12. *P. cardii.* A whole mount preparation of an individual fixed immediately after removal from a main duct of the digestive gland of a naturally fed *Cerastoderma edule*. The entire intestine shows an intense reaction for β-galactosidase; in the original preparation this can be seen to be localized in the contents of both the gut lumen and the intracellular vacuoles of the gastrodermis. The preparation was treated by the method for β-galactosidase described by Pearse (1972); the scale is 0.3 mm.

FIGURE 13. *Graffilla buccinicola.* A transverse section through the middle region of a mature individual dissected from the digestive gland of *Buccinum undatum*. The intestinal lumen contains cellular debris derived from the host's digestive epithelium and the gastrodermal cells show many heterogeneous inclusions resulting from phagocytosis of such debris. The section is stained by Mallory's trichrome method; the scale is 0.1 mm.

FIGURE 14. *G. buccinicola.* Part of a longitudinal section of an individual fixed immediately after removal from the digestive gland of *B. undatum* and showing a portion of the gastrodermis, the intestinal lumen, some vitellaria (v) and the epidermis. The intracellular inclusions (arrows) and vitellaria show medium and strong reactions, respectively, for β-glucuronidase. The section was treated for demonstration of β-glucuronidase by the method of Pearse (1972); the scale is 0.05 mm.

of *P. scrobiculariae*, however, dramatic increases occurred in the intensity of the reactions for these enzymes after *P. cardii* had fed *in situ* within its host and some individuals showed, additionally, strong positive reactions for β-galactosidase (Fig. 12). Specimens fed *in vitro* on enzymically inert foods did not develop such reactions and it is concluded that in this species, also, most of the enzymes responsible for digestion are derived from the host.

The food reserves resemble those of *P. scrobiculariae* and consist mainly of glycogen, supplemented by small amounts of lipid, laid down in the gastrodermis and mesenchyme.

Graffilla buccinicola

82.4% of the *Buccinum undatum* examined contained *Graffilla buccinicola;* the overall mean infection rate was 5.9 rhabdocoels per host with a range of 0 to 18 and a variance of 0.029. The rhabdocoel is hermaphrodite but not viviparous and specimens from both *B. undatum* and *Neptunea decemcostata* showed that the species is protandrous with considerable hypertrophy of the vitellaria

during the female phase. No significant differences in diet or gut structure were found in specimens from the two hosts; no observations on digestive enzymes could be made on those from *N. decemcostata,* however, as only Bouin-fixed material was available.

Structure of the gut. The gut is of the usual dalyellioid form but differs from that of the two species of *Paravortex* in that the pharynx is larger, with very well-developed musculature, and the gastrodermal cells lack the large intracellular fluid-filled vacuoles so characteristic of both *P. scrobicolariae* and *P. cardii.* The cells are monotypic, columnar, 75 to 100 μm tall and 30 to 50 μm wide, with basal nuclei and cytoplasm usually containing heterogeneous inclusions ranging in diameter from 2 to 20 μm (Fig. 13). These inclusions lie in vacuoles; their staining reactions vary enormously and they are very similar to those seen in cells of the hosts' digestive glands.

The food and feeding behavior. G. buccinicola lives permanently in the ducts and tubules of the digestive gland of *B. undatum* and was never found at other sites despite earlier reports (*e.g.,* Jameson, 1897; Dakin, 1912; Westblad, 1926) of its occurrence in the kidney and kidney ducts. Only the digestive gland of *N. decemcostata* was available for examination; the rhabdocoel was found in the ducts and tubules but Tomkiewicz and Morse (personal communication) report it also from the stomach of this host.

Feeding and digestion in the host B. undatum. B. undatum has carnivorous and scavenging habits and these are reflected in its digestive physiology, which is of the general pattern for carnivorous gastropods as reviewed by Owen (1966) and Purchon (1977). Gastric digestion is extensive and is followed by movement of soluble, largely proteinaceous, materials into the digestive gland. Here they are taken up by the cells of the tubules and digestion is completed intracellularly by non-specific esterases, A- and C-esterases, arylamidases, lipase and β-glucuronidase in a lysosomal sequence similar to that described for *S. plana* and *C. edule.* Acid phosphatase is present in the primary lysosomes, also, and the arylamidases occur in very large amounts. The heterolysosomes in which intracellular digestion proceeds have a great affinity for both acidic and basic stains and the residual bodies are usually rich in iron and calcium, as in *S. plana.* The digestive cells and tubules do not show cyclic activity, possibly because of the gastropod's irregular feeding habits; the residual bodies are eliminated by abstriction or, rarely, by cell disintegration and all stages of intracellular digestion can usually be found in adjacent tubules.

Diet and feeding in G. buccinicola. The rhabdocoel feeds in part on the host's semidigested food, as this passes into the digestive gland, and on discarded residual bodies. There are strong indications that it also takes in digestive cells from the tubule epithelium. The intestinal lumen often contains much cellular debris (Fig. 13) which is clearly derived from host digestive cells; the discrepancy between the large amounts regularly present and the observed low rate of disintegration of digestive cells suggests that the rhabdocoel is actively removing cells from the epithelium. This is supported by the presence in the intestinal lumen, and also intracellularly in the gastrodermis, of large host heterolysosomes still containing much protein and manifestly not yet in a condition suitable for discarding from their formative cells. Further support came from the observation

of damaged areas of tubule epithelium; these consisted of gaps three to four cells wide and in no way resembled the areas left by the normal shedding of cells seen in the bivalves studied.

G. buccinicola penetrates much farther into the host's digestive gland than do either *P. scrobiculariae* or *P. cardii* and is sometimes found within a digestive tubule pressed against the epithelium or, more rarely, outside the tubule proper but lying between adjacent tubules. This behavior is in accord with the postulated feeding on intact digestive cells and might account for the earlier reports of specimens from other sites in the body.

Digestion and food reserves. The gastrodermis of specimens maintained *in vitro* for up to 4 days showed only low levels of enzymic activity. The enzymes demonstrated were non-specific esterases, A- and C-esterases, lipase, arylamidases, β-glucuronidase, and acid and alkaline phosphatases. In contrast, others fixed directly on removal from the host showed very high levels of activity for all these enzymes, in both the lumen contents and the intracellular inclusions (Fig. 14). Unfortunately, specimens kept *in vitro* refused to feed on any type of enzymically inert food, so that it was impossible to determine whether or not this increased activity was endogenous or, as in *P. scrobiculariae* and *P. cardii,* of host origin. The latter seemed to be the more likely, though, taking into account the high level of activity in the lumen contents and the absence of any glandular components from the gastrodermis. Of the various enzymes demonstrated, arylamidases were particularly active; this was also the case in the host digestive cells, lending more support to the case for involvement of host enzymes in the rhabdocoel's digestive processes.

The food reserves resemble those of *P. scrobiculariae* and *P. cardii,* lipid being deposited sparingly in the gastrodermis and in some abundance in the vitellaria. Glycogen is abundant in the mesenchyme and especially so in the vitellaria. The latter also show intense reactions for non-specific esterases, lipase, acid and alkaline phosphatases and β-glucuronidase (Fig. 14) and are clearly sites of sustained metabolic activity.

Discussion

The salient point emerging from these observations is that the three entosymbiotic rhabdocoels studied show adaptive features in their nutritional physiology which indicate a definite trend toward parasitic habits.

The basic nutritional pattern in all three species would seem to be derived from a commensal type, since a proportion of their diet consists of the hosts' food; they do not, however, abstract this until it has undergone preliminary digestion by the hosts' gastric enzymes, and these continue to act within the flatworms' gut. This extension of a commensal habit to include utilization of the host's digestive enzymes could logically be expected to result in decreased production of enzymes within the symbiotes' own alimentary systems and to culminate, perhaps, in the loss of all endogenous digestive enzymes. The initial stages of this process are, in fact, found in all three species; gland cells producing extracellularly-acting proteases which are a common feature of many turbellarian alimentary systems (Jennings, 1974b) are absent and enzyme production in the monotypic gastro-

dermal cells is at a consistently low level. This contrasts sharply with the situation in rhabdocoels symbiotic with echinoderms, where the food consists of either co-symbiotes or the host's unaltered food and where the digestive physiology is very similar to that of free-living species (Jennings and Mettrick, 1968; Mettrick and Jennings, 1969; Jennings, 1977).

Since the rhabdocoels' digestive enzymes are supplemented by those of their hosts, it is not surprising that the types identified in their gut cells are appropriate to the diet of the particular host. *Graffilla buccinicola,* for example, from a carnivorous host, shows high levels of arylamidase activity during digestion as compared with the two species of *Paravortex* which come from omnivorous, largely herbivorous, hosts.

Utilization of the hosts' digestive enzymes has probably been facilitated by the peculiarities of the molluscan digestive system. The persistence in both bivalves and gastropods of extensive intracellular digestion within a specialized region of the gut, the digestive gland, provides a suitable feeding site for entosymbiotes away from the variable conditions of the stomach, but where products of gastric digestion, and gastric enzymes, are still available. Also, the elimination of unwanted end-products (spent or partially spent heterolysosomes) by either cell disintegration or abstriction provides a further source of both food and enzymes for the rhabdocoels. The digestive gland enzymes released during this elimination are believed to be important in the hosts' gastric physiology (Morton, 1956; 1967; Purchon, 1977) and so it is not surprising that they, too, are utilized by the symbiotes.

Use of cellular debris as food was probably the initial stimulus for the extension of the diet, in *Graffilla buccinicola,* to include intact host digestive cells. Here, the hyper-development of the pharynx relative to those of the two species of *Paravortex* is no doubt significant and *G. buccinicola* seems to be approaching the status of a tissue-feeding parasite of the type exemplified by some adult digenetic trematodes (Halton, 1967).

The iron contained in the residual bodies shed from the hosts' digestive glands and subsequently ingested by the rhabdocoels appears to be eliminated from the symbiotes' gastrodermis in soluble form, probably via the excretory system, and not by cell disintegration or abstriction. The ability to deal with the metal in this way is possibly another adaptation to entosymbiosis in molluscs and the particular types of food made available by this mode of life; the presence of iron in relatively large quantities was a consistent feature of the digestive glands of all four host species studied and may well be a characteristic of many other molluscs. It has been reported, for example, from the digestive glands of the bivalves *Nucula sulcata* (Owen, 1973) and *Mytilus edulis* (M. N. Moore, personal communication), and from populations of *Scrobicularia plana* widely separated, geographically, from those sampled in the present study (Bryan and Hummerstone, 1978; Bryan and Uysall, 1978).

Elimination of dietary iron in soluble form via the excretory system in the manner suggested for these entosymbiotic rhabdocoels is believed to occur also in certain sanguivorous digenetic trematodes (Jennings, 1968). This common ability to deal with excess dietary iron without cell disintegration, and the fact that the molluscan digestive gland remains the favored habitat of larval Digenea, lend

further support to the hypotheses that the Digenea arose from rhabdocoel-like ancestors and that the digestive gland is the primitive habitat of rhabdocoels living entosymbiotically in molluscs. Sanguivorous Monogenea, in contrast, do eliminate excess iron by cell disintegration (Halton and Jennings, 1965); these are typically ectoparasites of fishes and have no molluscan connections in their life cycles.

If the digestive gland is the primitive habitat, then the interesting migrations of *Paravortex scrobiculariae* between the gland and the intestine seem to be somewhat anomalous. The reasons for the migrations remain unknown, although they are presumably related in some way to the sharply defined feeding and digestive cycles of the particular host and hence, indirectly, to the tidal cycle. It may be that the ducts of the digestive gland are untenable at certain points in the host's digestive cycle, although, over the period in which the rhabdocoels are in the intestine (approximately mid-flood tide to mid-ebb tide), conditions would seem to be optimal for a stay in the gland. In the middle of this period, at high water, the host is feeding actively, food is passing into the stomach and beginning to enter the digestive gland and there should be maximum amounts of oxygen available, as considerable quantities of water are passing through the bivalve's filtration system. It is during the opposing phase, though, that *P. scrobiculariae* is to be found in the gland and, while the supply of partially digested food and cell debris is now maximal, the host's filtration system has closed down and oxygen is likely to be in short supply. In this situation, however, the adaptive significance of the haemoglobin present in this species becomes apparent. The pigment has been shown by spectrographic methods to be physiologically active and capable of reversible reactions with oxygen depending on the environmental oxygen tension (Phillips, 1978). Its concentration around the brain and pharynx suggests that it may well be important in supplying oxygen to these structures during feeding when the host is not filtering sea water and the oxygen tension of the mud habitat is very much reduced. The haemoglobin is obviously not essential for life under normal aerobic conditions, at least *in vitro,* specimens treated with carbon monoxide surviving for as long as untreated specimens. The carbon monoxide formed an apparently stable compound with the haemoglobin, judging from the permanence of the cherry red coloration such treatment induces, and this test is generally regarded as indicating whether or not an animal's haemoglobin is essential for survival (Prosser, 1973). The small amount of haemoglobin present would not seem to be capable of acting as an oxygen store for use over the period of feeding at low water, but it may be adequate for a process of facilitated diffusion of the type postulated by Wittenberg (1970). This would allow the rhabdocoel preferential use of such oxygen as is available, its host apparently lacking either haemoglobin or haemocyanin in common with most other bivalves (Morton, 1967). It would be interesting to attempt the carbon monoxide treatment *in vivo* in the host's natural habitat to test this hypothesis.

Paravortex cardii and *Graffilla buccinicola,* living in hosts whose habitats are permanently well-aerated and not subject to changes in oxygen tension, understandably lack haemoglobin.

The occurrence of haemoglobin in *P. scrobiculariae* is not unique in the Rhabdocoela. It has been reported, for example, in *Phaenocora unipunctata* and

P. typhlops (Crompton and Smith, 1963; Young and Harris, 1973) and both these species, significantly, are mud-dwellers living in conditions of low or variable oxygen tension. Another mud-dweller, the nematode *Enoplus brevis,* has a pharyngeal haemoglobin which presumably facilitates feeding, while its close relative *E. communis,* which lives in more aerated situations, does not (Atkinson, 1977).

The food reserves of all three species with their emphasis on glycogen storage, and the reproductive strategies of the two species of *Paravortex* with their emphasis on production of large numbers of offspring throughout the year, both conform to the pattern described for other entosymbiotic Platyhelminthes by Calow and Jennings (1974) and Jennings and Calow (1975). Very little information is available on the reproductive strategy of *G. buccinicola,* but the hypertrophy of its vitellaria, relative to those of free-living rhabdocoels, implies a similar emphasis on reproduction.

The peculiar supplementation of the nutrition of the embryo in the viviparous *P. scrobicolariae* and *P. cardii* by transfer of nutrients, sometimes particulate and undigested, from the parental gut is possibly a further adaptation to entosymbiosis. It may well enhance the chances of survival of the juveniles by allowing them to develop as far as possible, and certainly to a point where they can feed in the adult manner, before they leave the parent. Remarkable though this method of embryonic nutrition is, if it is accepted that entry of eggs or embryos into the gut for subsequent passage to the exterior via the mouth is an archaic turbellarian feature (*vide* Hyman, 1951), there is no reason why the embryos should not ingest gut contents during the process. On this view, transfer of nutrients to embryos still within the mesenchyme is simply an extension of the same phenomenon. There is, in fact, a precedent for unusual modes of embryonic nutrition in the genus *Paravortex;* Ball (1916) described uptake of free yolk globules by wandering amoeboid ectoderm and primary entoderm cells in gastrulae and postgastrulae of *P. gemellipara,* another viviparous species which is entosymbiotic in the bivalve *Modiolus,* and stated that the laden entoderm cells are then engulfed by the secondary entoderm. Ball does not mention any features in the nutrition of the advanced embryo comparable to those reported here, but the techniques used in his primarily developmental study would most probably have not revealed them.

Summary

1. Diet, gut structure, digestive physiology and food reserves have been studied in three entosymbiotic graffillid rhabdocoels.

2. In all three species these aspects of their nutritional physiology are much modified relative to those characteristic of free-living flatworms; they show adaptive features related to the entosymbiotic habit and, in particular, to the feeding and digestive processes of the respective hosts.

3. The three species feed on their hosts' partially digested food and the cellular debris released at the end of the digestive cycle occurring within the hosts' digestive gland. In one species this is extended to include removal of intact cells from the digestive epithelium.

4. The ingested food contains enzymes of host origin which continue to act within the rhabdocoels' alimentary systems and play a dominant role in the digestive physiology. There is concomitant reduction in the amounts of enzymes produced by the symbiotes.

5. Food reserves are of the type and relative amounts characteristic of other entosymbiotic Platyhelminthes and consist mainly of glycogen.

6. Two of the species studied are viviparous and there is evidence that advanced embryos receive nutrients from the parental gut.

7. One species, which migrates from the host's intestine into the digestive gland to feed during the mid-ebb to mid-flood tidal period, possesses an endogenous haemoglobin. It is suggested that this helps overcome oxygen deficiencies, during feeding, by facilitated diffusion. The other two species, which live and feed in conditions of more constant oxygen availability, lack haemoglobin.

LITERATURE CITED

Abe, M., S. P. Kramer, and A. M. Seligman, 1964. The histochemical demonstration of pancreatic-like lipase and comparison with the distribution of esterase. *J. Histochem. Cytochem.*, **12**: 364–383.

Atkins, D., 1934. Two parasites of the common cockle; a rhabdocoel *Paravortex cardii* Hallez and a copepod *Paranthessius rostratus* (Canu). *J. Mar. Biol. Assoc. U. K.*, **19**: 669–676.

Atkinson, H. J., 1977. The role of pharyngeal haemoglobin in the feeding of the marine nematode *Enoplus brevis* (Bastian). *J. Zool., (Lond.)*, **183**: 465–471.

Baer, J. G., 1971. *Animal Parasites.* Weidenfeld and Nicolson, London, 256 pp.

Ball, S. J., 1916. Development of *Paravortex gemellipara*. *J. Morphol.*, **27**: 453–557.

Bresciani, J., and M. Køie, 1970. On the ultrastructure of the epidermis of the adult female of *Kronborgia amphipodicola* Christensen and Kanneworff, 1964 (Turbellaria, Neorhabdocoela). *Ophelia*, **8**: 209–230.

Bryan, G. W., and L. G. Hummerstone, 1978. Heavy metals in the burrowing bivalve *Scrobicularia plana* from contaminated and uncontaminated estuaries. *J. Mar. Biol. Assoc. U. K.*, **58**: 401–419.

Bryan, G. W., and H. Uysal, 1978. Heavy metals in the burrowing bivalve *Scrobicularia plana* from the Tamar estuary in relation to environmental levels. *J. Mar. Biol. Assoc. U. K.*, **58**: 89–108.

Burstone, M. S., 1958. Histochemical demonstration of acid phosphatase with napthol AS-phosphates. *J. Natl. Cancer Inst.*, **21**: 523–539.

Burstone, M. S., and J. E. Folk, 1956. Histochemical demonstration of aminopeptidase. *J. Histochem. Cytochem.*, **4**: 217–226.

Calow, P. and J. B. Jennings, 1974. Calorific values in the phylum Platyhelminthes: the relationship between potential energy, mode of life and the evolution of entoparasitism. *Biol. Bull.*, **147**: 81–94.

Christensen, A. M., and B. Kanneworff, 1964. *Kronborgia amphipodicola* gen. et sp. nov., a dioecious turbellarian parasitizing ampeliscid amphipods. *Ophelia*, **1**: 147–166.

Christensen, A. M., and B. Kanneworff, 1965. Life history and biology of *Kronborgia amphipodicola* Christensen and Kanneworff (Turbellaria, Neorhabdocoela). *Ophelia*, **2**: 237–251.

Crompton, D. W. T., and M. H. Smith, 1963. Haemoglobin in a flatworm. *Nature, (Lond.)*, **197**: 1118–1119.

Dakin, W. J., 1912. *Buccinum. Liverpool Marine Biological Committee Memoirs*, Mem. XX. Williams and Norgate, London, 113 pp.

Hallez, P. 1909. Biologie, organisation, histologie et embryologie d'un rhabdocoele parasite du *Cardium edule* L., *Paravortex cardii* n. sp. *Arch. Zool. exp. gén.* Sér. 4, **9**: 1047–1049.

HALTON, D. W., 1967. Observations on the nutrition of digenetic trematodes. *Parasitology,* **57**: 639–660.

HALTON, D. W., AND J. B. JENNINGS, 1965. Observations on the nutrition of monogenetic trematodes. *Biol. Bull.,* **129**: 257–272.

HASSALL, M., AND J. B. JENNINGS, 1975. Adaptive features of gut structure and digestive physiology in the terrestrial isopod *Philoscia muscorum* (Scopoli) 1763. *Biol. Bull.,* **149**: 348–364.

HOLT, S. J., 1958. Studies in enzyme histochemistry. *Proc. R. Soc. Lond. B. Biol. Sci.,* **148**: 465–532.

HUGHES, R. N., 1969. A study of feeding in *Scrobicularia plana. J. Mar. Biol. Assoc. U. K.,* **49**: 805–823.

HYMAN, L. H., 1951. *The Invertebrates: Platyhelminthes and Rhynchocoela. The acoelomate Bilateria.* Volume II. McGraw-Hill Book Co., New York, 550 pp.

HYMAN, L. H., 1955. *The Invertebrates: Echinodermata. The coelomate Bilateria.* Volume IV. McGraw-Hill Book Co., New York, 763 pp.

JAMESON, H. L., 1897. Additional notes on the Turbellaria of the LMBC district. *Proc. Trans. Liverpool Biol. Soc.,* 11: 160–181.

JENNINGS, J. B., 1968. Nutrition and digestion. Pages 303–326 in M. Florkin and B. T. Scheer, Eds., *Chemical Zoology,* Volume II, Porifera, Coelenterata and Platyhelminthes. Academic Press, New York.

JENNINGS, J. B., 1971. Parasitism and commensalism in the Turbellaria. Pages 1–32 in B. Dawes, Ed., *Advances in Parasitology,* Volume IX. Academic Press, New York.

JENNINGS, J. B., 1974a. Symbioses in the Turbellaria and their implications in studies on the evolution of parasitism. Pages 127–160 in Winona B. Vernberg, Ed., *Symbiosis in the Sea.* University of South Carolina Press, Columbia, South Carolina.

JENNINGS, J. B., 1974b. Digestive physiology of the Turbellaria. Pages 173–197 in N. W. Riser and M. P. Morse, Eds., *Biology of the Turbellaria,* Libbie H. Hyman Memorial Volume. McGraw-Hill Book Co., New York.

JENNINGS, J. B., 1977. Patterns of nutritional physiology in free-living and symbiotic Turbellaria and their implications for the evolution of entoparasitism in the phylum Platyhelminthes. Pages 63–79 in T. G. Karling and M. Meinander, Eds., *The Alex Luther Centennial Syposium on Turbellaria. Acta Zoologica Fennica Volume 154,* Societas pro Fauna et Flora Fennica, Helsinki.

JENNINGS, J. B., AND P. CALOW, 1975. The relationship between high fecundity and the evolution of entoparasitism. *Occologia (Berl.),* **21**: 109–115.

JENNINGS, J. B., AND D. F. METTRICK, 1968. Observations on the ecology, morphology and nutrition of the rhabdocoel turbellarian *Syndesmis franciscana* (Lehman, 1946) in Jamaica. *Caribb. J. Sci.,* **8**: 57–69.

LLEWELLYN, J., 1965. The evolution of parasitic Platyhelminths. Pages 47–78 in Angela E. R. Taylor, Ed., *Evolution of Parasites,* Third Symposium of the British Society for Parasitology, Blackwell, Oxford.

MCQUISTON, R. W., 1969. Cyclic activity in the digestive diverticula of *Lasaea rubra* (Montagu) (Bivalvia: Eulamellibranchia). *Proc. Malacol. Soc. Lond.,* **38**: 483–492.

METTRICK, D. F., AND J. B. JENNINGS, 1969. Nutrition and chemical composition of the rhabdocoel turbellarian *Syndesmis franciscana,* with notes on the taxonomy of *S. antillarum. Can. J. Zool.,* **26**: 2669–2679.

MORTON, J. E., 1956. The tidal rhythm and action of the digestive system of the lamellibranch, *Lasaea rubra. J. Mar. Biol. Assoc. U. K.,* **35**: 563–586.

MORTON, J. E., 1967. *Molluscs.* Fourth Ed. Hutchinson and Co., London, 244 pp.

MORTON, J. E., A. D. BONEY, AND E. D. S. CORNER, 1957. The adaptations of *Lasaea rubra* (Montagu), a small intertidal lamellibranch. *J. Mar. Biol. Assoc. U. K.,* **36**: 383–405.

OWEN, G., 1966. Digestion. Pages 53–96 in K. M. Wilbur and C. M. Yonge, Eds., *Physiology of Mollusca,* Volume II. Academic Press, New York.

OWEN, G., 1972. Lysosomes, peroxisomes and bivalves. *Sci. Prog.,* **60**: 299–318.

OWEN, G., 1973. The fine structure and histochemistry of the digestive diverticula of the protobranchiate bivalve *Nucula sulcata. Proc. R. Soc. Lond. B. Biol. Sci.,* **183**: 249–264.

Owen, G., 1974. Feeding and digestion in the Bivalvia. Pages 1–35 in O. Lowenstein, Ed., *Advances in Comparative Physiology and Biochemistry,* Volume V. Academic Press, New York.

Pearse, A. G. E., 1972. *Histochemistry: theoretical and applied,* Third Ed. Churchill Livingstone, Edinburgh and London, 1518 pp.

Phillips, J. I., 1978. The occurrence and distribution of haemoglobin in the entosymbiotic rhabdocoel *Paravortex scrobiculariae* (Graff) (Platyhelminthes: Turbellaria). *Comp. Biochem. Physiol.,* (in press).

Prosser, C. L., 1973. Respiratory functions of blood. Pages 317–361 in C. L. Prosser, Ed., *Comparative Animal Physiology.* W. B. Saunders Co., Philadelphia.

Purchon, R. D., 1977. *The biology of the Mollusca,* Second Ed. Pergamon Press, Oxford, 560 pp.

Villot, M. A., 1879. Organisation et développement de quelques espèces de trématodes endoparasites marins. *Ann. Sci., Nat., Zool. Biol. Anim.,* Sèr. VI, **8**: 1–40.

Wahl, B., 1906. Untersuchungen über den Bau der parasitischen Turbellarien aus der Familie der Dalyelliiden (Vorticiden). *Sitzungsber. Oesterr. Akad. der Wiss., Math. Naturwiss. Kl.,* **115**: 417–473.

Westblad, E., 1926. Parasitische Turbellarien von der Westkuste Skandinaviens. *Zool. Anz.,* **67**: 323–333.

Wittenberg, J. B., 1970. Myoglobin-facilitated oxygen diffusion: role of myoglobin in oxygen entry into muscle. *Physiol. Rev.,* **50**: 559–636.

Young, J. O., and J. H. Harris, 1973. Another instance of haemoglobin in a flatworm *Phaenocora typhlops* (Vejdovsky 1880). *Freshwater Biol.,* **3**: 85–88.